PB-218 273

DEVELOPMENT OF A SELECTIVE ALGAECIDE TO CONTROL NUISANCE ALGAL GROWTH

Bernard L. Prows, et al

Dow Chemical Company
Freeport, Texas

January, 1971

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1 Accession Number	2 Subject Field & Group	SELECTED WATER RESOURCES ABSTRACTS INPUT TRANSACTION FORM
	04A	

5 Organization

The Dow Chemical Company
Texas Division
Freeport, Texas

6 Title

"Development of a Selective Algaecide to Control Nuisance Algal Growth"

10 Author(s)

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16 Project Designation

EPA, WQO Contract No. 14-12-814

21 Note

Details of illustrations in this document may be better studied on microfiche

22 Citation

23 Descriptors (Starred First)

*algaecides, * selective chemical control, *algal control, *nuisance algal control, Cyanophyta, laboratory assay, biocontrol.

25 Identifiers (Starred First)

*selective algaecide, *blue-green algae, *Anabaena, *Microcystis, test compound, phagocytic, selective screening, Ochromonas.

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A computer search was made of some 80,000 compounds and thirty-three were selected for testing. Those compounds which gave at least 80% control at 2.0 ppm against both Microcystis aeruginosa and Anabaena flos-aquae, the specified target species of blue-green algae, were selected for secondary screening tests at 1.0, 0.5 and 0.1 ppm.

Of the thirty-three compounds tested, four were selected for further research in the long-range algaecidal development program: 2,5-Dichloro-3,4-dinitrothiophene; 5-Chloro-2-(p-nitrophenoxy) phenyl phenyl iodonium chloride; 4-Amino-2,5-dibromophenylthiocyanate; and 1,1-Dimethyltetradecylamine hydrochloride.

In addition, an organism, Ochromonas ovalis, was discovered whose phagocytic action against Microcystis sp. was enhanced by compounds having algaecidal properties.

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Corvallis, OR 97330

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Freeport, Texas

for the

WATER QUALITY OFFICE

ENVIRONMENTAL PROTECTION AGENCY

Contract No. 14-12-814

January 1971

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Review Notice

This report has been reviewed by the Water Quality Office, Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Water Quality Office, Environmental Protection Agency.

ABSTRACT

The objective of this project was to discover a compound which would effectively and economically control the growth of nuisance species of blue-green algae and exert a minimum impact on the more desirable forms of life in the aquatic environment.

A computer search was made of some 80,000 compounds in Dow's "K-list". Thirty-three were selected for testing. Those compounds which passed the first screening test with at least 80% control at 2.0 ppm against both Microcystis aeruginosa and Anabaena flos-aquae, the specified target species of blue-green algae, were selected for secondary screening tests at 1.0, 0.5 and 0.1 ppm.

Of the thirty-three compounds tested, the following four were finally selected as candidates for further research through Phase II of the proposed long-range algaecidal development program: 2,5-Dichloro-3,4-dinitrothiophene; /5-Chloro-2-(p-nitrophenoxy) phenyl/ phenyl iodonium chloride; 4-Amino-2,5-dibromophenylthiocyanate; and 1,1-Dimethyltetradecylamine hydrochloride.

An additional major contribution of the project is the discovery of an organism, Ochromonas ovalis, whose phagocytic action against Microcystis sp. is enhanced in the presence of compounds having algaecidal properties.

In view of the foregoing, it is recommended that this project be continued into Phase II of the research and development of a selective algaecide to control nuisance algal growth.

This report was submitted in fulfillment of Project No. 14-12-814 between the Federal Water Quality Administration and The Dow Chemical Company.

Key Words: Test, Compound, Algae, Anabaena, Microcystis, Screen, Algaecidal, Ochromonas, Phagocytic, Activity.

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SECTION I

CONCLUSIONS

Of the thirty-three compounds which were tested for algaecidal activity, nineteen passed the test of 80% control at 2.0 ppm against *Microcystis aeruginosa* but only seven controlled *Anabaena flos-aquae* at the same concentration.

Those which passed the test at 2.0 ppm were tested further at 1.0, 0.5, and 0.1 ppm concentrations. Three compounds were finally selected through this process as candidates for Phase II of the long-range algaecide development program: 4-Amino-2,5-dibromophenylthiocyanate, [5-Chloro-2-(p-nitrophenoxy phenyl) phenyl] iodonium chloride, and 2,5-Dichloro-3,4-dinitrothiophene. A fourth compound (1,1-Dimethyltetradecylamine hydrochloride), whose activity against *Anabaena* was a little under the 80% level, is also recommended for further study due to the simplicity of its structure and the probability of discovering analogs to the parent compound which may have a higher activity rate.

A phagocytic organism, *Ochromonas ovalis*, was discovered which exhibited a high activity against *Microcystis*. In view of this discovery, together with the organism's enhanced activity in the presence of algaecidal compounds, the possibility of developing an effective and economical biological-chemical control system holds considerable promise.

SECTION II

RECOMMENDATIONS

In view of the results to this point, it seems advisable that this research program be continued into a second phase which would encompass a structure search for active analogs of the four selected compounds and an evaluation of algaecidal activity by controlled open-field testing.

Extended laboratory studies should be undertaken to determine such properties as herbicidal activity, fish and mammalian toxicities, compound stability, and formulation efficacy.

The possibility of developing an effective and economical biological-chemical control system should also be investigated further. Efforts should be directed toward optimization of Ochromonas and other phagocytic organisms as active agents against blue-green algae, in combination with chemical treatment.

SECTION III

INTRODUCTION

Over the past 50 years the use of potable water has spiraled from 30 to 150 gallons per person per day, and the total per capita consumption for all purposes is estimated at 1600 gallons per day (Cortell, 1970). According to the U. S. Geological Survey, in 1965 the total use of fresh water exceeded 310 billion gallons per day of which 23.6 billion gallons were consumed for domestic purposes. Accompanying the vital role which an adequate supply of water is playing, however, is the accelerating problem of excessive nutrification.

During the process of aging, a lake undergoes changes and the normal process of maturation takes place. Runoff from the watershed contributes nutrients which support the vegetative growth of phytoplankton and the littoral vegetation. Eventually the vegetative growth becomes great enough to fill in the lake and extinction occurs. The process of nutrient enrichment is called "eutrophication", the speed of which is dependent upon the amount of nutrients washing off the watershed and into the lake. Under natural conditions, unaffected by man, the input of nutrients in runoff and in precipitation is usually low. However, with the impact of man and his culture, the speed is enormously increased. Developments such as cultivation, agriculture and the establishment of cities accelerate nutrient input. Excessive quantities of nutrients cause abnormal growth of algae and aquatic weeds which seriously affects the quality and use of domestic water supplies.

Although a number of elements are essential for algal growth, the principal elements of concern are nitrogen and

phosphorus. Some species of algae such as Anabaena have the ability to fix atmospheric nitrogen (Fitzgerald, 1968; Jackson, 1967), but most species require its presence in the form of nitrates and ammonia.

The major sources of nitrogen and phosphorus are from sewage outfalls, industrial plants and runoff from watersheds and agricultural lands. MacKenthun (1969) lists sewage and industrial effluents as accounting for about two-thirds of the total phosphorus load dumped into our domestic water supplies each year.

There are obvious benefits of eutrophication, such as increasing the growth of vegetative life in the water, thus furnishing additional food and protection for fish and other forms of animal life. In fact, the intentional addition of nutrients to farm ponds to increase fish production is a frequently recommended practice in many parts of the United States (Davison, 1955).

However, even in farm ponds, overfertilization can cause such dense algal growths that the ecological balance of the pond is upset and the support of desirable fish life becomes limited. Katz and Gaufin (1953) found that the appearance of large numbers of trash fish often occurs under such conditions. Aesthetic values may be lowered, for those who wish to use the body of water for recreational purposes, because of obnoxious odors and the presence of floating algal scums.

Even though the photosynthetic action of algae is beneficial in removing carbon dioxide from the water and supplying oxygen to it, an overabundance of algae can cause serious ecological problems because of oxygen depletion due to respiratory activity at night and because of

decaying algae. Numerous instances have been reported in which heavy algal blooms have resulted in large fish kills due to oxygen depletion in the water (Keup et al, 1967). During periods of heavy cloud cover and long nights, the oxygen level in a lake or pond may fall below the level of survival for certain species of fish (Jackson, 1967; MacKenthun et al, 1964).

Further, a number of algal species is known to have toxic properties. MacKenthun and Ingram (1967) listed twenty outbreaks of human gastrointestinal disorders, fourteen recorded human respiratory disorders and ten human skin disorders, all associated with algae. Numerous cases of cattle being killed by ingestion of large quantities of algae-infected water have been reported. However, since the likelihood of human consumption is minimal, Gorham (1964), who reviewed the literature on toxic algae as a public health hazard, concluded that the fish and livestock poisonings produced by algal waterblooms should be considered as nuisances and economic hazards rather than public health hazards.

Several approaches to eutrophication problems are possible, and a number of these is being investigated in the National Eutrophication Program. Basically, the approaches fall into four broad categories: (1) mechanical, (2) biological, (3) ecological, and (4) chemical. Mechanical approaches involve, chiefly, the design and engineering of machines for harvesting algae and aquatic weeds. Biological approaches have included the use of viruses, insects, algae-eating fish and other organisms. Ecological approaches have included diversion of nutrient-rich water around the lake, flushing with nutrient-poor water, nutrient removal at the sewage treatment plant, and nutrient removal from the lake itself by crops or other processes.

Chemical approaches for eutrophication control have not been investigated thoroughly even though a great deal of effort has been expended in other aspects of pest control. One of the reasons for this is the difficult problem encountered in obtaining a material which will kill nuisance algae or control its reproduction without at the same time adversely affecting desirable organisms in the aquatic environment. Copper sulfate has been used most widely as a chemical means for controlling algal growths. However, copper is not biodegradable, and copper salts will accumulate on lake bottoms following repeated treatments (Nichols et al, 1964). Hasler (1947) and Kunkel (1969) point out the possible deleterious effects of this accumulation on lake ecology.

There is, at present, no registered chemical available which is effective against the target species of algae in surface waters from which drinking water may be prepared.

In recent years a considerable amount of effort has been expended in search of a chemical to replace copper sulfate that would be more effective, non-accumulative, and perhaps even less costly over a longer period of time (MacKenthun et al, 1964).

Although it is likely that no single approach will serve in all cases, it is reasonable that a chemical approach to the control of nuisance algal growths might be suitable for many situations, particularly when the algae have gotten out of control and a balance needs to be restored.

SECTION IV

EXPERIMENTAL PROCEDURES

The prime objective of the project was to discover a compound which would control the growth of various species of blue-green algae economically and have a minimum impact on other forms of life in the aquatic environment.

The approach to the problem involved initially making a computer search of some 80,000 compounds in Dow's K-list in order to select those having the highest probability of measuring up to the established criteria for an ideal algaecide. Most of these compounds had already been tested against several species of algae, fish and some terrestrial plants and animals. A final hand selection was then made from the computer printout, eliminating those compounds which had inherent or demonstrated undesirable properties, such as high fish, terrestrial plant, or mammalian toxicities. Finally, the candidate compounds were divided into several priority groups according to a selection criteria.

The following criteria were established for selection of the candidate test compounds.

Priority Group No. 1

Compounds in this group were tested against Oscillatoria or some other species of blue-green algae and exhibited an activity of 90% of better at 2.0 ppm or less or were highly algaestatic. These compounds also possessed the following characteristics:

1. Known activity against other algae and aquatic weeds - preferable.

2. Low level of fish toxicity - mandatory.

3. Low level of mammalian toxicity - mandatory.

4. Low level of terrestrial plant toxicity - highly preferable.

5. Composition including no heavy metals, such as copper, silver, arsenic, or mercury, or components which would be likely to make the cost economically unfeasible.

Priority Group No. 2

Compounds in this group demonstrated an activity of 90% or better against at least one species of blue-green algae at concentrations between 2.0 and 10.0 ppm and met all other criteria.

Priority Group No. 3

Compounds in this group were not tested on blue-green algae but only on Chlorella (green algae), exhibited an activity of 90% or better at 2.0 ppm or less, and met all other criteria.

Thirty-three compounds were thus selected--nineteen in Group No. 1, eight in Group No. 2, and six in Group No. 3. Each of these compounds were then tested, in order of priority, against the two target species of blue-green algae--Anabaena flos-aquae and Microcystis aeruginosa--specified by the Federal Water Quality Administration.

The primary screening tests involved the testing of each compound against established standard stock cultures of the two blue-green algal species at 2.0 ppm concentration. Those which passed the primary test at 80% activity or

better against both species of algae were retained for testing through the secondary screening test at 1.0, 0.5 and 0.1 ppm chemical concentrations. The final selection was made from those which passed the secondary screening test at 80% activity or better against both Microcystis and Anabaena.

Equipment

A metal framework composed of 1 1/2-inch by 2-inch slotted angle iron was constructed for support of the waterbath shakers and the overhead fluorescent lighting assembly used in the primary and secondary screening tests.

The supporting framework was 30 inches by 50 inches in lateral dimensions and 54 inches high. Two waterbath shakers were supported on 3/4-inch plywood, mounted on the framework 36 inches below the fluorescent lights. (See Figure 1.)

The lighting intensity was attenuated and adequately diffused by interposing several layers of cheesecloth between the four 48-inch, 25-watt, cool white fluorescent bulbs and the test flasks.

Algal Cultures

Uni-algal cultures of Anabaena flos-aquae and Microcystis aeruginosa were obtained from the Pacific Northwest Water Laboratory, Corvallis, Oregon. The algae were received in good condition and were immediately transferred into sterile Gorham's medium, adjusted to a pH of 8.5. Gorham's medium, as used throughout this project, consisted of the following components in milligrams per liter of distilled water: $NaNO_3$ - 496, $MgSO_4 \cdot 7H_2O$ - 49, $CaCl_2 \cdot 2H_2O$ - 36, Na_2CO_3 - 20, $NaSiO_3$ - 58, ferric citrate - 6, EDTA - 1 (Hughes et al, 1958; Fitzgerald, 1968). In view of the expected pH ranges of

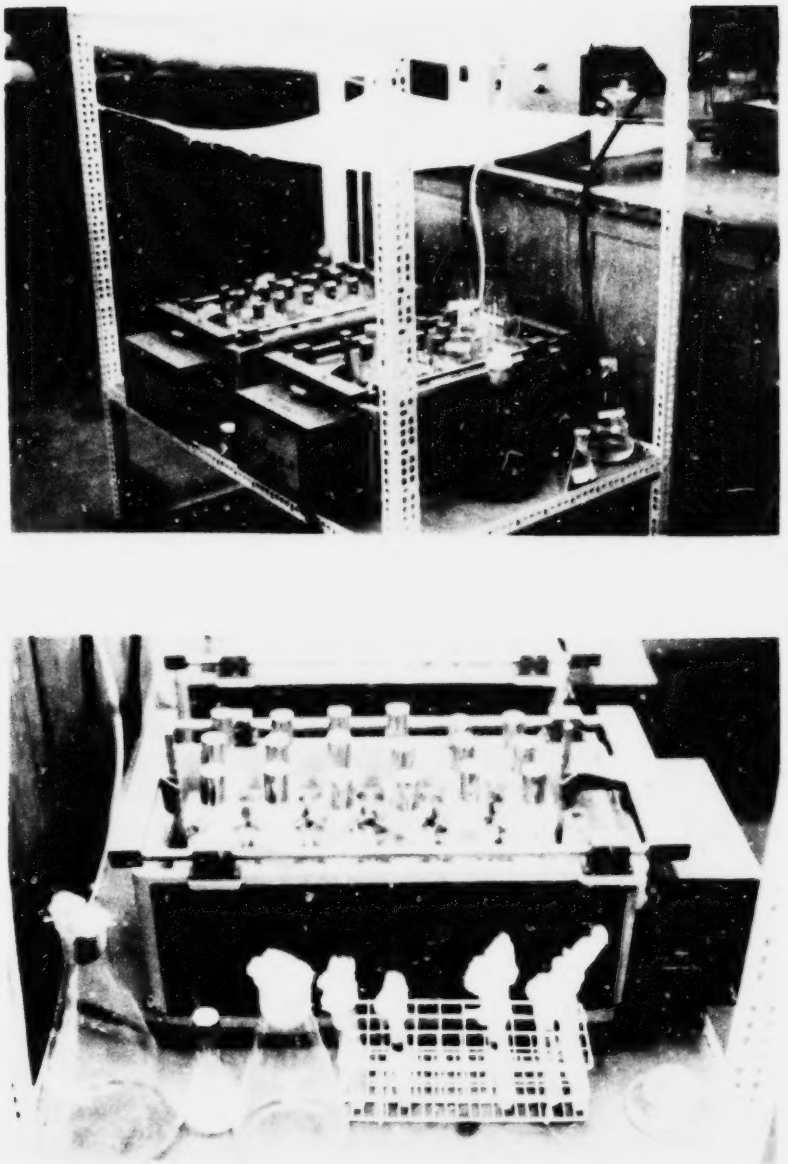


Figure 1

Waterbath Shakers and Lighting Assembly
Used for Screening Tests

most fresh inland waters and the increased solubility of nutrients at a lower pH, the medium was later adjusted to a pH of 7.5 - 8.0 for all stock cultures and tests (Kuentzel, 1969).

Algal stock cultures were kept under static conditions at about 100 foot-candles of cool-white fluorescent light. Under these conditions the cultures maintained a normal, healthy appearance and growth pattern. In Figure 2, the magnified healthy algae are shown.

Test Compounds

Samples of each of the compounds were ordered from Dow's storage vault at Midland, Michigan. Test compounds were formulated just prior to the initiation of the screening tests in order to avoid the possibility of compound degradation.

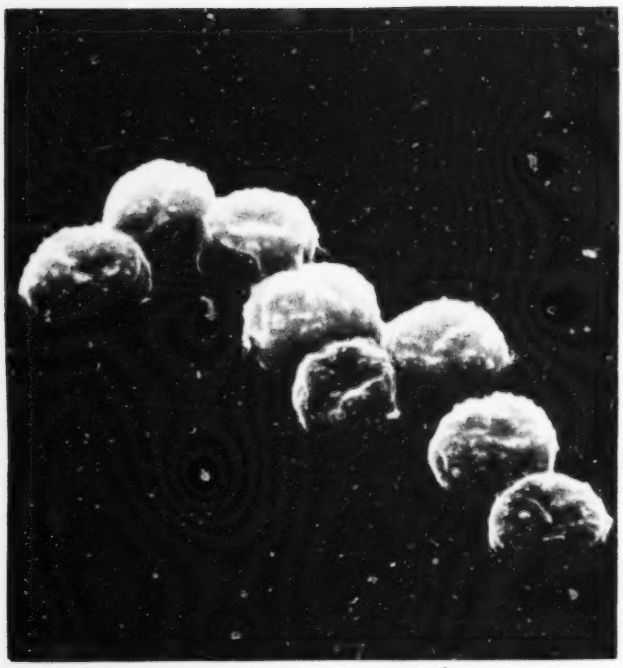
Experimental Design

Duplicate test flasks were used for screening with one control for each algal species, test chemical, and concentration. The tests were prepared by adding 26 ml of test chemical, 3 ml of 10X Gorham's nutrient concentrate, and 1.0 ml standard algal innoculum to each of the 125-ml Erlenmeyer test flasks.

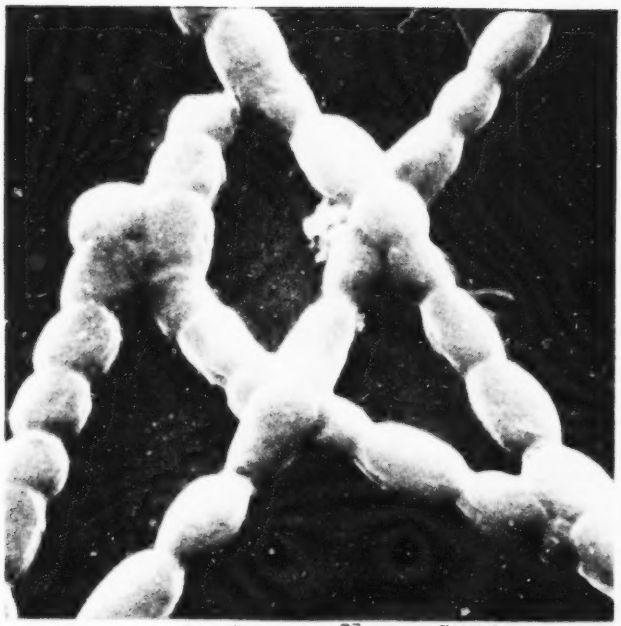
Initial cell counts were determined and relative intensity readings taken with the fluoro-microphotometer. The extreme sensitivity of fluorescent measurements allowed for *in vivo* determinations of algal culture densities without making chlorophyll extractions (Bain, 1969). The flasks were then constantly agitated in waterbath shakers at 80 excursions per minute under continuous illumination as recommended in the Provisional Algal Assay Procedure (Joint Industry/Government Task Force on Eutrophication, 1969). Cell growth was monitored daily by visual inspection.

Figure 2

ALGAL TEST SPECIES



Microcystis aeruginosa
5000X



Anabaena flos aquae
5000X

Final cell counts and relative intensity readings were taken at the end of the 7-day test periods.

Compound activity was specified as percent of growth suppression:

$$\% \text{ Activity} = \left(1 - \frac{\text{Final test flask cell count}}{\text{Final control cell count}}\right) \times 100$$

Due to the filamentous nature of Anabaena, relative intensity readings, rather than cell counts, were used to assess cell growth, together with visual inspection and biomass determinations.

Formulation

Test chemicals were formulated using an acetone-emulsifier (AE) solvent system whenever possible. The acetone-emulsifier was comprised of two surfactants, SPAN and TWEEN, in acetone at 2,000 ppm and 500 ppm concentrations, respectively.

Preparation of Test Flasks

In order to help eliminate undue shock to algal cells and optimize compound solubility, it was felt that the test chemical solutions should be made up in the desired concentrations before nutrifying with "10X" Gorham's medium concentrate and standard algal inocula. To accomplish this, 30 mg of test chemical was dissolved in 20 ml acetone-emulsifier, plus 80 ml distilled water. This formed a 300-ppm test compound concentrate. A 5-ml aliquot of this concentrate was then added to 645 ml sterile, distilled water to make a 2.3-ppm test chemical solution. Thus, when 26 ml of this solution was combined with 3.0 ml Gorham's medium 10X concentrate and 1.0 ml standard algal inoculum,

the final test chemical concentration in the test flask was 2.0 ppm, and the algal nutrients were diluted to the correct concentration. All primary screening tests were run at 2.0 ppm.

Test chemicals for the secondary screening tests were formulated in the same manner and then diluted to the desired 1.0, 0.5 or 0.1 ppm concentrations with distilled water just prior to the preparation of the test flasks.

Standard Inocula

The standard algal inocula was prepared by centrifuging and washing 7-day-old cultures two times with sterile Gorham's medium and then making a final resuspension of 30×10^6 cells per ml. In the case of Anabaena cultures, the resuspension was gauged to a relative intensity reading equivalent to that obtained with a 30×10^6-cell/ml. culture of Microcystis. For more accurate standard algal determinations, a small aliquot of cell concentrate was removed and diluted 1:10 with Gorham's medium. The relative intensity readings taken on the diluted suspensions were then used to gauge the proper cell concentrations of the standard inocula. This was done to help eliminate non-linearity error due to cell-shielding in highly concentrated cultures. Thus, 1.0 ml of standard algal inoculum (30×10^6 cells/ml), added to 29 ml of test compound solution, gave an initial cell count of 1.0×10^6 cells/ml or its equivalent to each test flask.

Compound Screening Tests

For the primary screening at 2.0 ppm, six compounds were tested simultaneously against each algal test species under identical conditions of light, temperature and agitation. Tests were prepared in duplicate with one control for each

chemical concentration and algal species. Tests and controls were prepared in standard 125-ml. culture flasks, utilizing specially designed stainless steel closure caps equipped with expanded polystyrene inner cushions to eliminate test flask contamination from outside sources and still allow for gas exchange between the flasks and the outside atmosphere.

Initial cell counts and relative intensity readings were taken at the onset of each testing series. Visual observations of cell growth were recorded each day during the particular test period. At the end of the 7-day period, the tests were terminated, and final cell counts, relative intensity readings, and visual and microscopic observations were recorded. Compound activities were then calculated on the basis of the number of cells killed in the test flasks compared to the number of viable cells existing in the controls.

Biomass Determinations

For the purpose of cross-checking the methods used for assessing cell growth, it seemed desirable to make biomass determinations using cultures of the target algae and to correlate the results with cell counts and relative intensity readings taken on the same cultures.

Two 50-ml aliquots were taken from a Microcystis culture which was in its logarithmic growth phase and were filtered through 0.45 μ Millipore® filters which had been previously desiccated for 24 hours, then weighed. After filtering, the samples were oven-dried for two hours and then desiccated for two hours, before the final weighings. An identical procedure was followed using 50% and 25% dilutions of the original stock culture. The average number of cells per

gram of dry weight was calculated as well as the cell mass per million cells and the cell density per relative intensity unit. Cell numbers were thus convertible to dry weight equivalents which were related to the relative intensity readings taken with the fluoro-microphotometer (Table I).

TABLE I

BIOMASS DETERMINATIONS - MICROCYSTIS AERUGINOSA

Spl. No.	RI[1]	Cell Count (Cells/ml)	Spl. Vol. (ml)	Total Cell Count	Dry Sample Weight	Cells/Gram	Cell Mass[2] Gms/10^6 Cells	Cell Density RI Unit
I	2.0	10.35×10^6	50	51.75×10^7	.0056	9.20×10^{10}	1.085×10^{-5}	5.2×10^6
II	1.12	5.73×10^6	50	29.65×10^7	.00295	9.51×10^{10}	1.070×10^{-5}	5.1×10^6
III	.060	2.70×10^6	50	13.50×10^7	.00165	8.25×10^{10}	1.225×10^{-5}	4.5×10^6

[1]Fluorometric Relative Intensity

[2]Dry Weight

SECTION V

DISCUSSION

Compound Activity

A total of thirty-three compounds were tested for algaecidal activity through the primary screen at 2.0 ppm against the two target algal species, Microcystis aeruginosa and Anabaena flos-aquae. Nineteen of these passed the test at 80% activity or better against Microcystis but only seven passed the test against Anabaena. The testing results are summarized in Table II.

Those compounds which passed the first screen test against both test species of blue-green algae were selected for further testing at 1.0, 0.5 and 0.1 ppm concentrations. Compound No. K-25,148 (2,5-Dichloro-3,4 dinitrothiophene) exhibited the greatest activity of any of the compounds tested. The activity against Microcystis was 100%, in concentrations as low as 0.1 ppm and 87% against Anabaena at 1.0 ppm.

Other compounds selected through the secondary screening tests as candidates for Phase II testing included No. K-54,925 (4-Amino-2,5-dibromophenyl-thiocyanate) and No. K-53,179 /5-Chloro-2,p-(nitrophenoxy phenyl) phenyl/7 iodonium chloride. One additional compound, No. K-30,339 (Dimethyl-tetradecyl-amine hydrochloride), is also recommended for additional study due to its simple structure and relatively high activity against Microcystis, even though it fell slightly below the 80% activity level against Anabaena. The chemical structures of the four candidate compounds are shown in Figure 3.

Of the two species of blue-green algae tested, Anabaena has rather consistently proven to be the most resistant to chemicals exhibiting algaecidal properties.

TABLE II

ACTIVITIES OF COMPOUNDS AT VARIOUS CONCENTRATIONS
AGAINST TWO SPECIES OF BLUE-GREEN ALGAE

K-Number	Name of Compound	Percent Activity							
		Microcystis aeruginosa Concentration in ppm				Anabaena flos-aquae Concentration in ppm			
Group No. 1		2.0	1.0	0.5	0.1	2.0	1.0	0.5	0.1
K-24,816	3,3',4',5-Tetrachlorosalicylanilide	100	27	I	I	100	41	I	I
K-30,598	4-Hydroxy-1-naphthyl thiocyanic acid ester	83	-	-	-	I	-	-	-
K-43,413	5,5'-Dichloro-2,2'-dihydroxybenzophenone	95	-	-	-	41	-	-	-
K-45,179	3,Tert-butyl-4-hexylphenyl thiocyanic acid ester	85	-	-	-	I	-	-	-
K-48,501	3,7-Dichlorophenoxiodinin hydrogen sulfate	73	-	-	-	33	-	-	-
K-53,780	1,1-Dimethyl-2-(α,α,α,-trifluro-p-tolyl) urea	91	75	63	I	70	12	I	I
K-53,910	2-Benzyl-5,6-dimethyl-benzimedizole	100	-	-	-	50	-	-	-
K-54,925	4-Amino-2,5-dibromophenyl thiocyanate	100	100	100	I	100	84	47	45
K-54,928	4-Amino-2,6-dichlorophenyl thiocyanate	100	-	-	-	14	-	-	-
K-24,810	3,4,5-Trichloro-o-creosol	36	-	-	-	11	-	-	-
K-27,970	3,4,5',5,6-Pentachloro-2,2'-methylenediphenol	100	99	99	31	86	30	I	26
K-50,569	4-Cyclohexyl-o-cresol	47	-	-	-	18	-	-	-

Table II (Continued)

K-Number	Name of Compound	Percent Activity: Microcystis aeruginosa Concentration in ppm 2.0	1.0	0.5	0.1	Anabaena flos-aquae Concentration in ppm 2.0	1.0	0.5	0.1
K-50,904	Phenyl-2,3,3-trichloro-allyl acetic acid ester	I	-	-	-	I	-	-	-
K-50,985	(p-Phenoxyphenyl)-iodonium phenyl trichloroacetate	96	-	-	-	I	-	-	-
K-53,179	[5-Chloro-2-(p-nitrophe-noxy) phenyl] phenyl iodonium chloride	100	98	90	60	100	85	58	48
K-53,336	2-Imino-α,α'-diphenyl hydrochloride-1,3-benzimidazolinediethanol	I	-	-	-	I	-	-	-
K-53,947	bis-(2,3-Epoxy propyl) ether	32	-	-	-	I	-	-	-
K-48,153	Tetrahydrodithio-3-thio-phene carbamic acid	I	-	-	-	I	-	-	-
K-55,564	n-Benzoyl glycinonitrile	I	-	-	-	I	-	-	-
Group No. 2									
K-11,651	2,2'-Thiobis(4,6-dichloro-phenol)	92	-	-	-	50	-	-	-
K-13,837	1-Fluoro-2,4-dinitrobenzene	22	-	-	-	50	-	-	-
K-25,148	2,5-Dichloro-3,4-dinitro-thiophene	100	100	100	100	90	87	46	I
K-30,339	1,1-Dimethyltetradecyl-amine hydrochloride	100	100	100	I	98	71	36	33
K-30,341	1,1-Dimethyl-9-octadecenyl-amine hydrochloride	100	100	I	I	85	33	25	25

Table II (Continued)

K-Number	Name of Compound	Percent Activity: Microcystis aeruginosa, Concentration in ppm 2.0	1.0	0.5	0.1	Anabaena flos-aquae, Concentration in ppm 2.0	1.0	0.5	0.1
K-48,719	m-Diacetylbenzene	I	-	-	-	60	-	-	-
K-48,689	Phenoxyiodinin-fluoro-phosphate	89	-	-	-	I	-	-	-
K-50,922	Thio-m-(trifluoromethyl)-S-p-nitrophenyl carbonilic acid ester	85	-	-	-	40	-	-	-
Group No. 3									
K-19,372	Trichlorocresol	I	-	-	-	31	-	-	-
K-20,041	3-Diallyl-2,4-dihydroxy-benzophenone	13	-	-	-	11	-	-	-
K-20,575	n-Dodecyl-n,n-diethyl-1,3-propane-diamine hydro-bromide	100	100	100	I	67	I	I	I
K-46,884	3,3'-Cyclohexylidene-bis-(2-methoxy-p-phenyl-eneazo)-bis-4,6-dihydroxy-2-naphthalenesulfonic acid	I	-	-	-	24	-	-	-
K-48,542	Phenyltrichloromethyl sulfide	100	-	-	-	I	-	-	-
K-51,818	1,2-di-o-tolylhydrazine	12	-	-	-	37	-	-	-

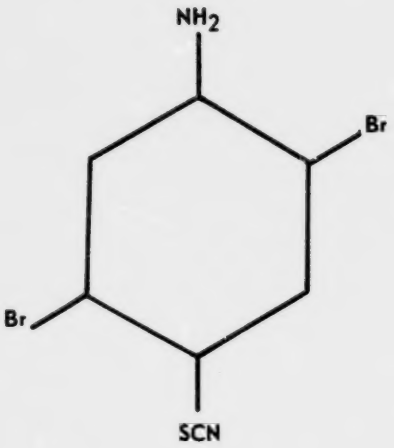
NOTE: I = Inactive (<10% activity)

Figure 3

K–54,925

4–Amino–2,5–dibromophenyl
–thiocyanic acid ester

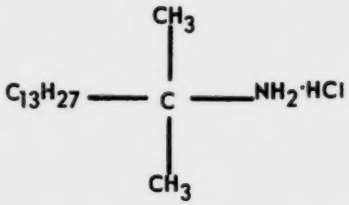
$C_7H_4Br_2N_2S$



K–30,339

1,1–Dimethyltetradecylamine
–Hydrochloride

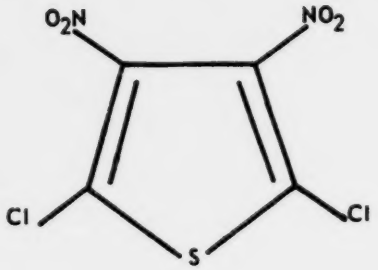
$C_{16}H_{35}N \cdot HCl$



K–25,148

Dichloro–3,4 dinitrothiophene

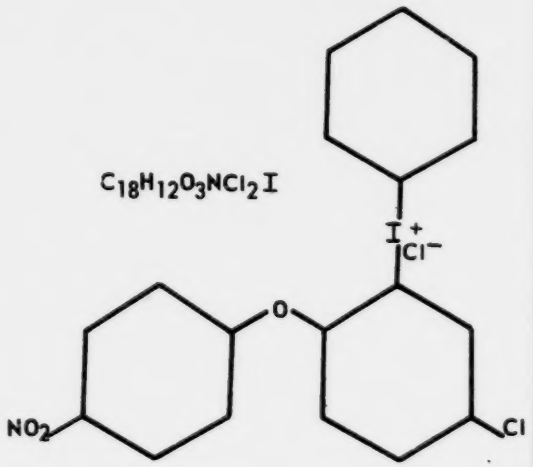
$C_4Cl_2N_2O_4S$



K–53,179

[5–Chloro–2–(p–nitrophenoxy)phenyl]
–phenyl Iodonium chloride

$C_{18}H_{12}O_3NCl_2I$



Phagocytic Organism

During the course of the project, an extraneous organism was discovered which exhibited severe phagocytic activity against Microcystis. It was observed that the presence of this organism caused a rapid decline in the viability of the Microcystis stock cultures (Figure 4 and Table III).

With the aid of electron micrography and in corroboration with Dr. M. J. Wynne of the University of Texas, the organism was identified as Ochromonas ovalis (Figure 5), a golden brown, biflagellated algae with a small, laterally positioned chloroplast. The organism was found to have low photosynthetic ability and phagocytic activity which varied with temperature and environmental conditions (Table III).

There is an apparent anomaly between the end results of the experiments involving the action of Ochromonas on Microcystis at the different temperatures. In the first case at 24°C and 100 foot-candles, the Ochromonas cell density reached its maximum in about 2 1/2 days. About the same time the Microcystis count approached zero (Figure 4). In the latter case at 6°C and 120 foot-candles, the phagocytic activity rate of Ochromonas was greatly reduced, and the Microcystis cell count did not approach depletion until 7 days.

The relative growth patterns of the two algal species, however, were about the same with the Ochromonas reaching its maximum about the time the Microcystis were depleted. At the lower light intensity and normal temperature (40 foot-candles, 24°C), the cell depletion was more rapid, but the Ochromonas had started to reduce in number from its maximum before all of the Microcystis were gone.

Figure 4

OCHROMONAS OVALIS VERSUS MICROCYSTIS AERUGINOSA

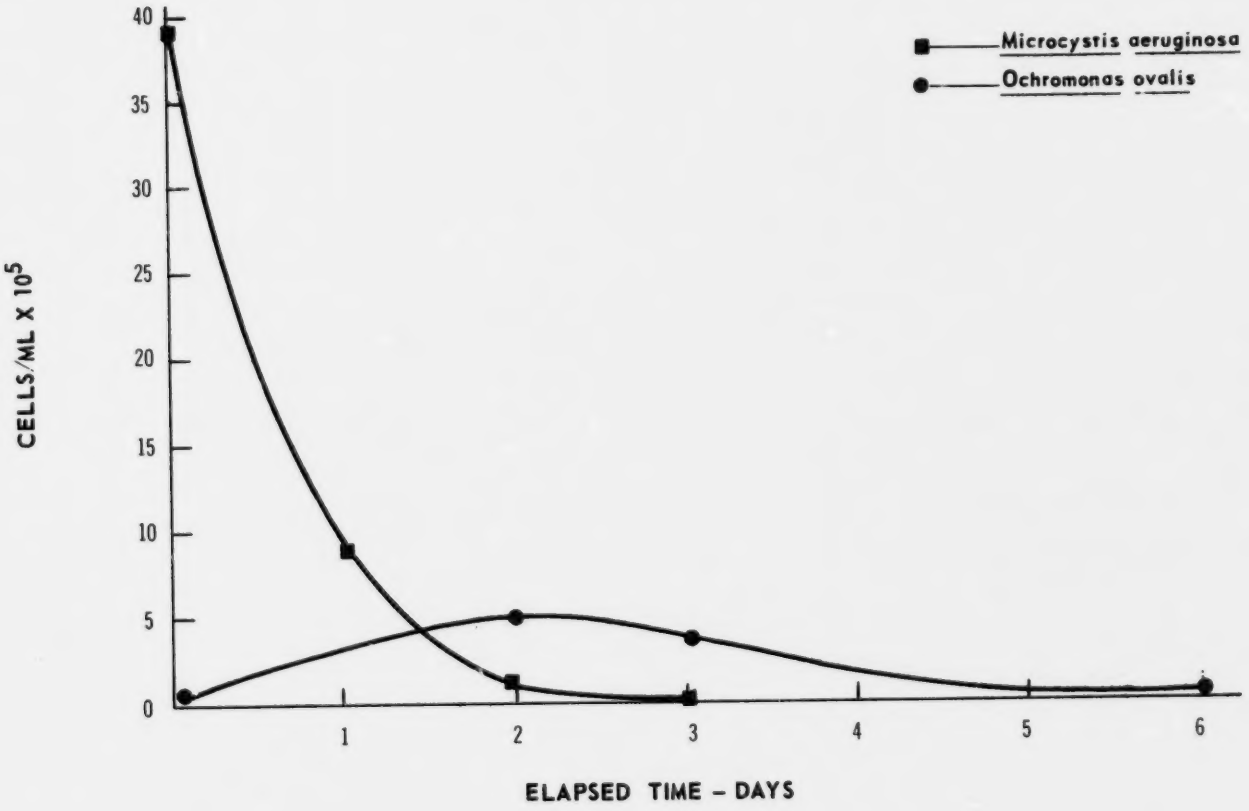


TABLE III

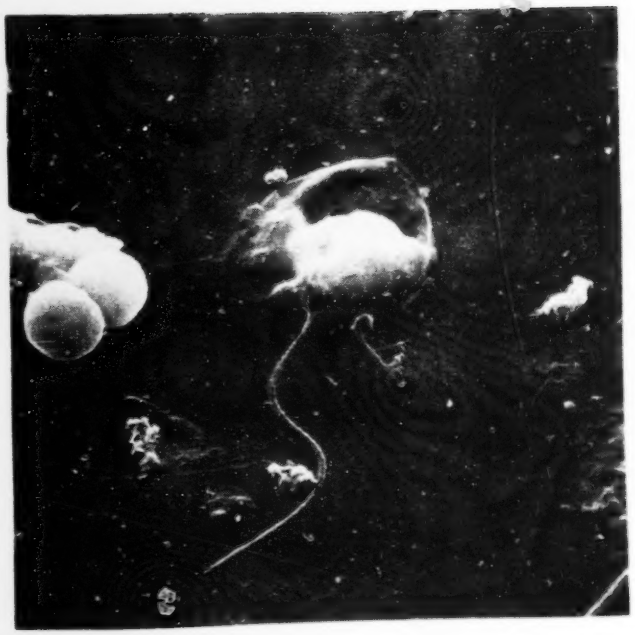
PHAGOCYTIC ACTIVITY OF OCHROMONAS

(Comparison of Cell Concentrations as Functions of Temperature and Time)

Elapsed Time	6°C		24°C	
	Microcystis (Cells/ml)	Ochromonas (Cells/ml)	Microcystis (Cells/ml)	Ochromonas (Cells/ml)
0	4.4×10^6	6.0×10^4	4.4×10^6	6.0×10^4
1	5.0×10^6	7.0×10^4	1.8×10^6	18×10^4
3	3.7×10^6	24×10^4	0.13×10^6	57×10^4
7	0.1×10^6	50×10^4	0.10×10^6	50×10^4
	120 foot-candles		40 foot-candles	

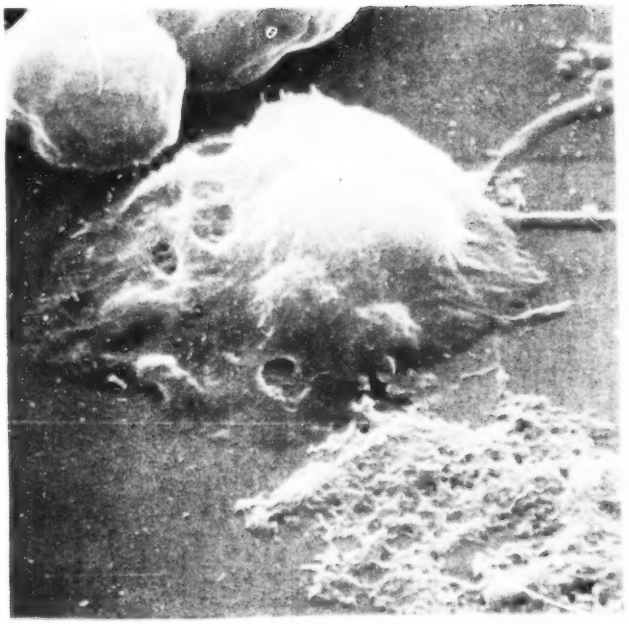
Figure 5

CONTAMINANT ORGANISM



Ochromonas ovalis

Scanning Electron Micrograph
5000X



Ochromonas ovalis

Scanning Electron Micrograph
10,000X

The shifting of the curves to the right is due to a reduction of metabolic activity by a factor of two for each 10°C reduction in temperature. The growth pattern difference between cultures reared at two different light intensities (Figure 4 and Table III) indicates that light may influence phagotropy, perhaps through regulation of the organism's net energy requirements.

It is noteworthy to observe that the organism's growth and activity against Microcystis were somewhat enhanced when in the presence of some of the test chemicals used in the primary screening tests. As seen in Table IV, although the difference in cell depletion of Microcystis was not great, the higher cell density of Ochromonas was significant. Thus, if large volumes of Microcystis were increasingly available, the activity of Ochromonas against them should show greater differences as a function of time.

Because of culture decline due to the presence of the contaminant organism, new stock cultures of Microcystis were obtained from several different sources and used for further experimental work with Ochromonas as well as the formal compound screening tests.

After filtering the contaminated culture through a 0.45-micron Millipore® filter under sterile conditions and inoculating the filtrate with a new, healthy inoculum, it was found that new "clean" Microcystis cells grew well in the filtrate but declined rapidly in the presence of the contaminant organism. (See Table V and Figure 4.) Thus, the culture depletion appeared to be due to "grazing" of the contaminant organisms rather than the presence of a toxin being produced by them.

TABLE IV

COMPARISON OF ALGAL DENSITIES
IN THE PRESENCE OF K-43,413

Time (Days)	Cells/ml With Test Compound		Cells/ml Without Test Compound	
	Microcystis	Ochromonas	Microcystis	Ochromonas
0	3.0×10^6	0.05×10^4	3.0×10^6	0.05×10^4
1	2.5×10^6	1.1×10^4	1.4×10^6	1.1×10^4
2	0.0×10^6	15×10^4	0.01×10^6	6.2×10^4
3	0.0×10^6	18×10^4	0.00×10^6	16×10^4
4	0.0×10^6	11.5×10^4	0.00×10^6	9.8×10^4

Conditions:

Temperature - 24°C
Light - 100 foot-candles
Agitation - 80 excursions/min.
Compound Conc'n - 2.0 ppm

TABLE V

COMPARISON OF GROWTH RATES OF *MICROCYSTIS*

(Determination of Media Toxicity)

Time (Days)	Media Used for Culturing *Ochromonas** (Cells/ml)	Control (New Media) (Cells/ml)
0	1.0×10^6	1.0×10^6
1	1.4×10^6	1.3×10^6
2	1.8×10^6	1.7×10^6
3	2.0×10^6	2.1×10^6
6	2.3×10^6	2.5×10^6

*Filtered through 0.45-micron Millipore® filter.

A rather extensive literature search was made to determine whether Ochromonas sp. had been reported as having demonstrated phagocytic activity against blue-green algae. Although considerable work has been done on the vitamin requirements of Ochromonas (Reason, 1954; Prescott, 1970; Reason, 1956) and some mention was made of their phagocytic nature (Lewin, 1962), no reports were found concerning Ochromonas exhibiting phagocytic activity against blue-green algae.

It was found that the presence of this contaminant organism was also the main factor contributing to anomalous relative intensity readings obtained with the fluoro-microphotometer while monitoring culture growths. Algal cultures contaminated with Ochromonas gave relative intensity readings three to five times greater than the controls. It appeared as though this could be due to a breakdown of Chlorophyll "a" to Phaeophytin "a", but a comparison of the relative absorption and emission spectra in the literature did not bear this out (Yentsch and Menzel, 1963; Holm-Hansen et al, 1966). It was determined that the high relative intensity values were due to the golden brown pigment present in the contaminant organism which had a much higher excitation-emission factor than Chlorophyll "a", the primary pigment in blue-green algae (Table VI).

"Clean-Up" Procedures

Through a series of tests using various antibiotics, suggested by Vance (1966) for selective cleaning of algal cultures, and other chemical agents, an effective clean-up procedure was developed to rid the cultures of the contaminant organism. Neomycin®, Chloromycetin® and Actidione® proved to be either ineffective against Ochromonas or met with only marginal success. Potassium dichromate, however, when added

TABLE VI

FLUOROMETRIC INTENSITIES OF ALGAL CULTURES

Organism	Cell Count (Cells/ml)	R.I. Readings
Microcystis	1.86×10^6	0.86
Ochromonas	50×10^4	3.95
Microcystis Filtrate	-	0.002
Ochromonas Filtrate	-	0.005

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to contaminated cultures of Microcystis proved to be selective against the unwanted organism and many types of bacteria when used at 200 ppm concentrations for 3 to 4 hours. (See Table VII.)

After centrifuging and washing the culture several times, the Microcystis seemed to have suffered only slight damage from the treatment. Later the treatment was modified to 500 ppm $K_2Cr_2O_7$ for 15 minutes which seemed to be adequate to kill the extraneous organisms and was less severe on the algal cultures.

Culturing Optimization

The optimal rearing conditions are believed to be: (1) use of Gorham's medium, made from 10X concentrates of the individual components and adjusted to a pH of 7.7 to 8.0 (Figures 6 and 7); (2) eighty excursions per minute agitation rate (Figure 8); (3) continuous, cool white fluorescent light at 120 foot-candles intensity (Figure 6); (4) an initial cell concentration of 1.0×10^6 cells per milliliter in the test flasks; and (5) a temperature of 24°C ±1°C.

Activity Comparison

Copper sulfate ($CuSO_4 \cdot 5H_2O$) was used to compare the activities of the compounds being tested. The activities determined for copper sulfate, together with the four other candidate test compounds chosen for additional study, are compiled in Table VIII. All of the four selected compounds had higher activities against Microcystis at equivalent concentrations than did hydrated copper sulfate. Two of the compounds had activities at 1.0 ppm which were greater than copper sulfate against Anabaena.

TABLE VII

TOXICITY OF POTASSIUM DICHROMATE TO ALGAL CULTURES

Treatment Time (Hours)	$K_2Cr_2O_7$ Concentration			
	50 ppm	100ppm	200 ppm	500 ppm
1/2	P*	P*	P*	F
1	P*	P*	P*	P
2	P*	P*	G	P
4	P*	P*	G	D
24	F	P	D	D

*Ochromonas still present and viable.

NOTE:

Relative Condition of Microcystis:

G = Good
F = Fair
P = Poor
D = Dead

Figure 6

GROWTH OF MICROCYSTIS AS A FUNCTION OF LIGHT INTENSITY

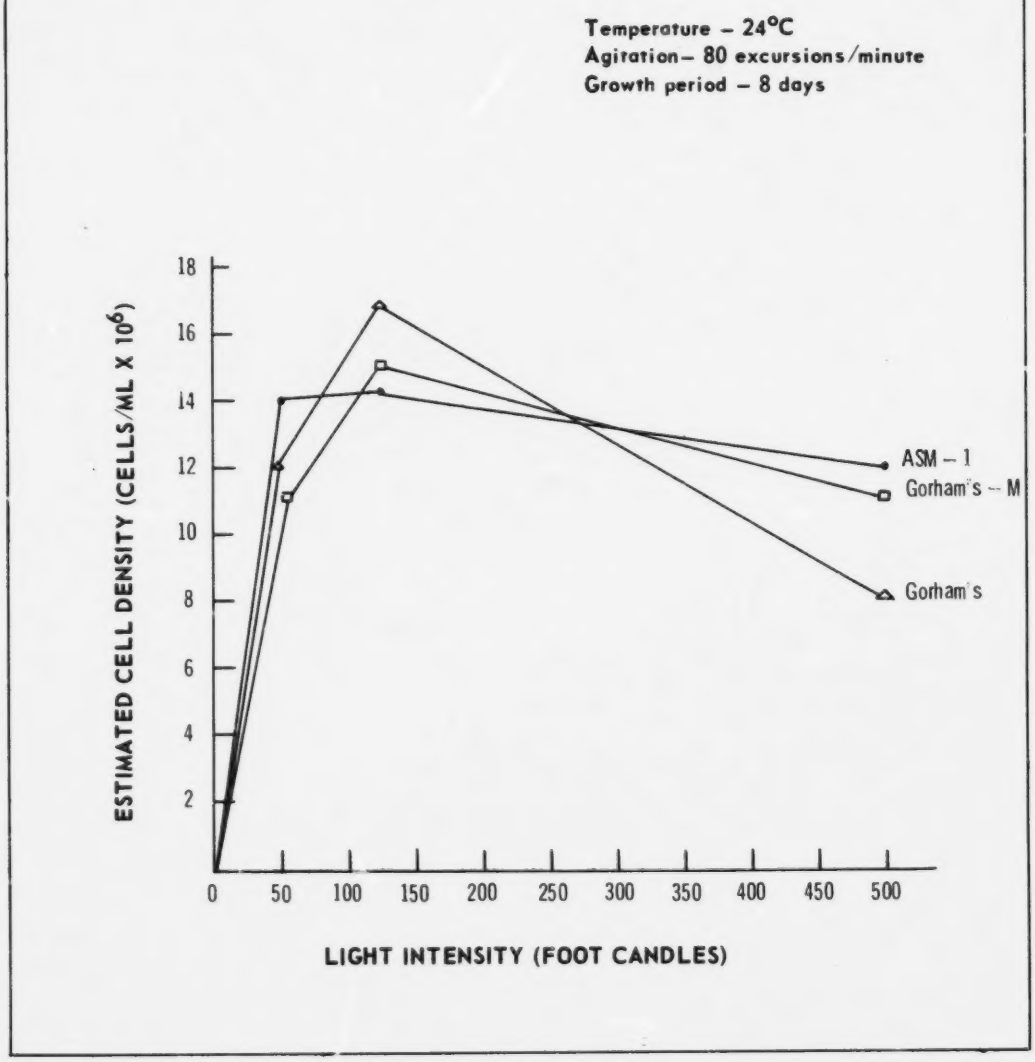

Figure 7

GROWTH OF MICROCYSTIS AS A FUNCTION OF AGITATION RATE

Temperature – 24°C
Light Intensity – 120 foot candles
Gorham's medium

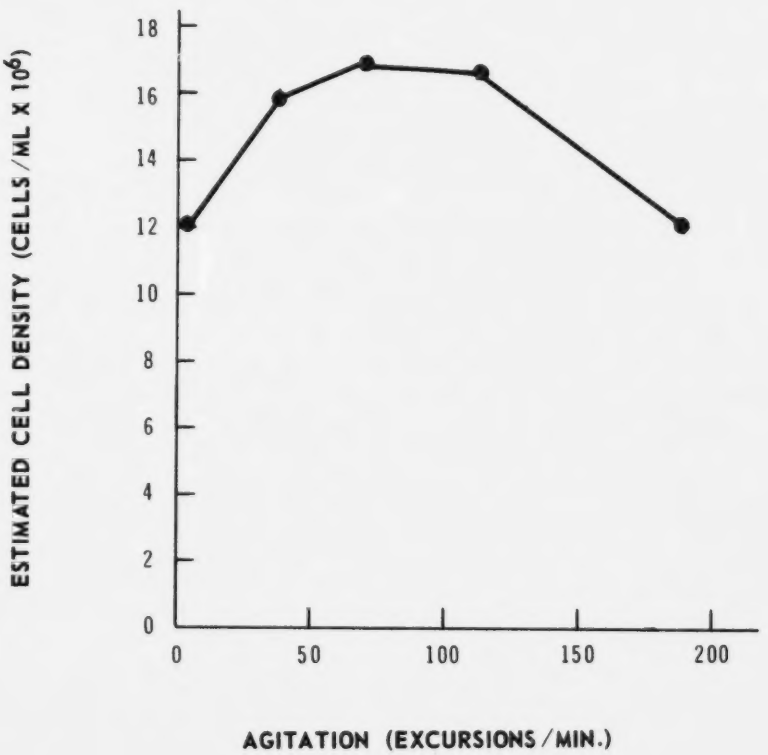

Figure 8

GROWTH OF MICROCYSTIS AERUGINOSA AT VARIOUS pH VALUES

Light intensity– 100 foot candles
Agitation – 80 excursions/minute
Temperature – 24° C.

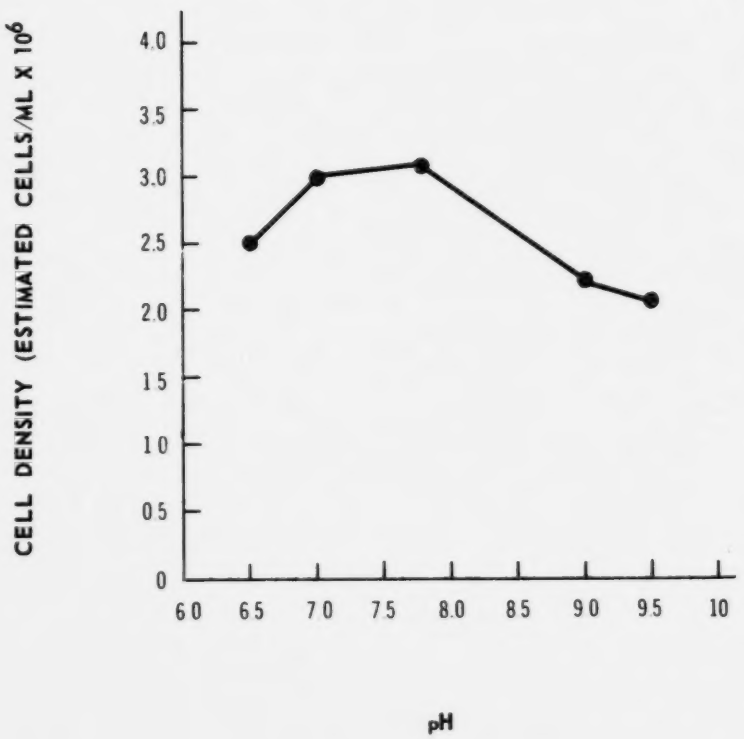

TABLE VIII

SELECTED COMPOUNDS VERSUS COPPER SULFATE

Name of Compound	Percent Activity							
	Microcystis aeruginosa Concentration in ppm				Anabaena flos-aquae Concentration in ppm			
	2.0	1.0	0.5	0.1	2.0	1.0	0.5	0.1
2,5-Dichloro-3,4-dinitrothiophene	100	100	100	100	90	87	46	I
4-Amino-2,5-dibromophenyl thiocyanate	100	100	100	I	100	84	47	45
[5-Chloro-2-(p-nitrophenoxy) phenyl] phenyl iodonium chloride	100	98	90	60	100	76	58	48
1,1-Dimethyl tetradecylamine hydrochloride	100	100	100	I	98	71	36	33
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	100	99	55	12	95	82	I	I

NOTE: I = Inactive (<10% activity)

Cost Estimate

One of the prime considerations in the search for a selective algaecide to control nuisance algal growth is the economic factor. A particular test compound, even though it may have demonstrated high activity against the target algae and may look promising from a safety and selectivity standpoint, may still be an unrealistic choice if it is clearly beyond the realm of economic feasibility. It is therefore desirable to make some projected estimates of economic limitations of the candidate compounds even at this early stage of development.

A relative judgment of the economics may be made by using the activity data for the candidate compounds and for copper sulfate ($CuSO_4 \cdot 5H_2O$) because, at the present time, it is the most widely used algaecide (Otto and Bartley, 1966).

For control of Microcystis and most filamentous algae such as Anabaena, the dosage generally recommended is 2 to 4 pounds of copper sulfate per acre-foot of water. Since the relative algaecidal resistance of Anabaena is greater than Microcystis, as was consistently verified by the screening tests, a copper sulfate dosage of 4.0 and 3.0 pounds per acre-foot, respectively, is used. An estimate of the dosage quantities of each of the four selected candidate compounds for comparison is tabulated in Table IX.

TABLE IX

ESTIMATED QUANTITIES OF SELECTED COMPOUNDS
NEEDED FOR ALGAL CONTROL

Name of Compound	Required Levels (Lbs/Acre-Foot)*	
	Microcystis	Anabaena
Copper sulfate ($CuSO_4 \cdot 5H_2O$)	3.0	4.0
2,5-Dichloro-3,4-dinitrothiophene	<0.50	3.8
4-Amino-2,5-dibromophenyl thiocyanate	1.6	3.9
/5-Chloro-2-(p-nitrophenoxy) phenyl/ phenyl iodonium chloride	1.8	4.3
1,1-Dimethyltetradecylamine hydrochloride	1.6	4.6

*$CuSO_4 \cdot 5H_2O$ taken as standard of comparison.

SECTION VI

REFERENCES

Bain, R. C., Jr.: "Algal Growth Assessments by Fluorescence Techniques", Proceedings of the Eutrophication Biostimulation Assessment workshop, U. S. Department of the Interior, Federal Water Pollution Control Administration. (1969)

Bartsch, A. F.: "Biological Aspects of Stream Pollution", Sewage Works Journal, 20:292-302. (1948)

Bartsch, A. F. and W. M. Ingram: "Stream Life and the Pollution Environment", Public Works, 90:104-110. (1959)

Bueltman, C. G. (Chairman): "Provisional Algal Assay Procedure", Joint Industry-Government Task Force on Eutrophication. (1969)

Cortell, J. M.: "The Role of Herbicides in the Preservation of our Urban and Industrial Water Resources", Weeds, Trees, and Turf, June: 12-28. (1970)

Davis, V. E.: "Managing Farm Fish Ponds", Farmers Bulletin No. 2094, U. S. Department of Agriculture. (1955)

Gorham, P. R.: "Toxic Algae as a Public Health Hazard", Journal of American Water Works Association, 56 (11): 1481-1488. (1964)

Hasler, A. D.: "Antibiotic Aspects of Copper Treatment of Lakes", Wis. Acad. Sci., Arts & Lett., 39:97-103. (1947)

Holm-Hansen, O. et al: "Fluorometric Determination of Chlorophyll", Institute of Marine Resources, Scripps Institute of Oceanography. (1966)

Jackson, Daniel F.: "Algae, Man and the Environment", Syracuse University Press, Syracuse, N. Y. (1967)

Katz, M. and A. R. Gaufin: "The Effects of Pollution on the Fish Population of a Midwestern Stream", Trans. Am. Fish Soc., 82:156-165. (1953)

Keup, L. E., Ingram, W. M. and Mackenthun, K. M.: "Biology of Water Pollution", U. S. Department of the Interior, Federal Water Pollution Control Administration, June. (1968)

Kuentzel, L. E.: "Bacteria, Carbon Dioxide and Algal Blooms", J. W. P. C. F., 41:10, 1737-1747. (1969)

Kunkel, D. H.: "Algae Control in Ponds", Farm Pond Harvest, Summer. (1969)

Lewin, R. A.: "Physiology and Biochemistry of Algae", Academic Press, New York, N. Y. (1962)

MacKenthun, K. M.: "The Practice of Water Pollution Biology", U. S. Department of the Interior, Federal Water Pollution Control Administration. (1969)

MacKenthun, K. M. and W. M. Ingram: "Limnological Aspects of Recreational Lakes", U. S. Department of Health, Education and Welfare. (1964)

MacKenthun, K. M. and W. M. Ingram: "Biological Associated Problems in Freshwater Environments--Their Identification, Investigation and Control", U. S. Department of the Interior, Federal Water Pollution Control Administration. (1967)

Middlebrooks, E. J., T. E. Maloney, C. F. Powers and L. M. Knack: "Proceedings of the Eutrophication-Biostimulation Assessment Workshop", Sanitary Engineering Research Lab., University of California and U. S. Dept. of the Interior, Federal Water Pollution Control Administration. (1969)

Nichols, M. S., T. Henkel and D. McNall: "Copper in Lake Muds From Lakes of the Madison Area", Trans. Wis. Acad. Sci., Arts & Lett., 38:333-350. (1946)

Otto, N. E. and T. R. Bartley: "Aquatic Weed Control Studies", U. S. Department of the Interior, Bureau of Reclamation, Research Report No. 2. (1966)

Prescott, G. W.: "How to Know the Fresh Water Algae", Wm. C. Brown Publishing Company. (1970)

Reazin, G. H., Jr.: "On the Dark Metabolism of Golden Brown Alga, Ochromonas Malhamensis", Am. Jour. Bot., 41:9, 771-777. (1954)

Reazin, G. H., Jr.: "The Metabolism of Glucose by the Alga Ochromonas Malhemensis", Plant Phys., 31:4, 299-303. (1956)

Smith, G. M.: "The Fresh Water Algae of the United States", McGraw-Hill Book Company, New York, N. Y. (1950)

Vance, B. D.: "Sensitivity of Microcystis Aeruginosa and Other Blue-Green Algae and Associated Bacteria to Selected Antibiotics", I. Phycol. 2:125-128. (1966)

Vance, B. D. and Smith, D. L.: "Effects of Five Herbicides on Three Green Algae", Texas Journal Science, 20:4, 330-335. (1969)

Yentsch, C. S. and D. W. Menzel: "A Method for Determination of Phytoplankton Chlorophyll and Phaeophytin by Fluorescence", Deep Sea Research, 10:221-231. (1963)

Zajic, J. E.: "Properties and Products of Algae", Plenum Press, New York. (1970)